Translation

PCT/EP2003/008199

(31114)

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference M/43191-PCT | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No. PCT/EP2003/008199 | International filing date (*day/month/year*) 25 July 2003 (25.07.2003) | Priority date (*day/month/year*) 26 July 2002 (26.07.2002) |

International Patent Classification (IPC) or national classification and IPC
C12P 23/00

Applicant
BASF AKTIENGESELLSCHAFT

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 25 February 2004 (25.02.2004) | 10 December 2004 (10.12.2004) |
| Name and mailing address of the IPEA/EP<br><br>Facsimile No. | Authorized officer<br><br>Telephone No. |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP2003/008199

## I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-25, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the claims:

pages _____, as originally filed

pages _____, as amended (together with any statement under Article 19

pages _____, filed with the demand

pages 1-19, filed with the letter of 05 October 2004 (05.10.2004)

☒ the drawings:

pages 1/5-5/5, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1-15, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, Nos. 20

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP 03/08199

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-19 | YES |
| | Claims | | NO |
| Inventive step (IS) | Claims | 1-19 | YES |
| | Claims | | NO |
| Industrial applicability (IA) | Claims | 1-19 | YES |
| | Claims | | NO |

2. Citations and explanations

1. Reference is made to the following documents:

D1: SCHOEFS A B B et al.: "Astaxanthin accumulation in *Haematococcus* requires a cytochrome P450 hydroxylase and an active synthesis of fatty acids", FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, Vol. 500, No. 3, 6 July 2001 (2001-07-06), pages 125-128, XP004251391, ISSN 0014-5793

D2: US 2002/051998 A1 (SCHMIDT-DANNERT CLAUDIA et al.), 2 May 2002 (2002-05-02)

D3: DE 100 51 175 A (BASF AG), 2 May 2002 (2002-05-02)

D4: SCHMIDT-DANNERT CLAUDIA: "Engineering novel carotenoids in microorganisms", CURRENT OPINION IN BIOTECHNOLOGY, Vol. 11, No. 3, June 2000 (2000-06), pages 255-261, XP002261192, ISSN 0958-1669

2. The amendments submitted with the letter of 5 October 2004 meet the requirements of PCT Article 34(2)(b).

3.1 Document D1 discloses the oxidation of β-carotene to astaxanthin in *Haematococcus* and provides evidence that cytochrome P450 is involved in the oxidation process.

3.2 Document D2 shows that the bacterial monooxygenases cytochrome P450 BM3 and P450CAM (not from *Thermus sp.*) can be used to oxidise various metabolites, such as carotenoids and terpenoids.

3.3 Document D3 discloses the isolation and cloning of the CYP175A1 cytochrome P450 gene from *Thermus thermophilus*. Various possible substrates for the cytochrome P450 monooxygenases are disclosed, such as ionones, which are terpene compounds; however, there is no mention of carotenoids.

3.4 Document D4 discloses the engineering of carotenoid biosynthesis enzymes and discusses the assembling of various of these (crt) genes to generate new metabolic pathways. Cytochrome P450 enzymes from *Thermus sp.* are not disclosed.

4.1 Taking D3 to be the closest prior art, the problem addressed by the present invention is that of providing a new use for the enzyme from *Thermus sp.*

4.2 The solution to the problem involves the oxidation of carotenoid substrates, and is not obvious because it is not apparent to a person skilled in the art, either from D3 alone or from a combination of D3 with D1, D2 or D4, that carotenoids can be substrates for the P450 cytochrome monooxygenase isolated from *Thermus sp.*

4.3 Claims 1 to 19 therefore meet the requirements of novelty, inventive step and industrial applicability (PCT Article 33(2), 33(3) and 33(4)).